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IMMUNOLOGIC CROSS-REACTIVITY BETWEEN ANIMAL TISSUE AND ENTEROBACTERIAL COMMON ANTIGEN (CA)

FINAL REPORT

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The purpose of the current study was to (1) assess the immunogenicity of enterobacterial common antigen (CA) in the guinea pig as measured by cutaneous delayed by hypersensitivity and humoral antibody response to CA; (2) determine whether protection against infectious challenge, as measured by survival, is evoked in mice immunized with erythrocytes treated with CA; (3) assess strains of out-bred, in-bred and gnotobiotic mice for a tissue antigen cross-reactive with CA as measured by the capacity of tissue extracts to modify RBC for hemagglutination and to inhibit CA hemagglutination. CA engendered both humoral and cellular responses in guinea pigs. Swiss white albino mice immunized with heterologous erythrocytes treated with CA are significantly, albeit transiently, protected against infectious challenge with a CA producing enteropathogen. An antigen which cross-reacts with CA was demonstrated in the spleens, livers, or kidneys of Swiss white albino, CS7BL/6HA, and DBA mice. Likely, the reactions observed by hemagglutination-inhibition and rabbit-immunization protocols do not reflect contamination by enteric microbial flora; tissues excised from gnotobiotic Swiss white albino mice produced similar patterns of cross-reactivity with CA.

It is concluded that many questions must be resolved relative to CA and its influence in immunity and disease. Chief among these questions is the relationship CA has to normal or healthy animal and human tissue. A clear definition of this relationship may help us understand the capricious influence Gram-negative enteric microorganisms have on animals and man. With this understanding, we will have better recourse to effective management of subjects at risk.

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FINAL REPORT

IMMUNOLOGIC CROSS-REACTIVITY BETWEEN ANIMAL TISSUE AND ENTEROBACTERIAL COMMON ANTIGEN (CA)

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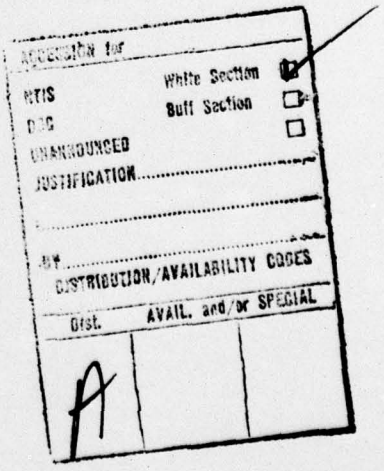

SUMMARY

The purpose of the current study was to (1) assess the immunogenicity of enterobacterial common antigen (CA) in the guinea pig as measured by cutaneous delayed type hypersensitivity and humoral antibody response to CA; (2) determine whether protection against infectious challenge, as measured by survival, is evoked in mice immunized with erythrocytes treated with CA; (3) assess strains of outbred, in-bred and gnotobiotic mice for a tissue antigen cross-reactive with CA as measured by the capacity of tissue extracts to modify RBC for hemagglutination and to inhibit CA hemagglutination. CA engendered both humoral and cellular responses in guinea pigs. Swiss white albino mice immunized with heterologous erythrocytes treated with CA are significantly, albeit transiently, protected against infectious challenge with a CA producing enterogathogen. An antigen which cross-reacts with CA was demonstrated in the spleens, livers, or kidneys of Swiss white albino, CS7BL/6HA, and DBA mice. Likely, the reactions observed by hemagglutination-inhibition and rabbit-immunization protocols do not reflect contamination by enteric microbial flora; tissues excised from gnotobiotic Swiss white albino mice produced similar patterns of cross-reactivity with CA.

It is concluded that many questions must be resolved relative to CA and its influence in immunity and disease. Chief among these questions is the relationship CA has to normal or healthy animal and human tissue. A clear definition of this relationship may help us understand the capricious influence Gram-negative enteric microorganisms have on animals and man. With this understanding, we will have better recourse to effective management of subjects at risk.

FORWARD

All research proposed by the Principal Investigator and sponsored by the U.S. Army Medical Research and Development Command under Contract DADA 17-73-C-3047 has been addressed. The purpose of the proposed research has been accomplished; 10 reprints of each of the 6 publications (see Literature Cited, page 11) resulting from this research were submitted earlier to the Contracting Officer in compliance with requirements. Written permission has been obtained from each of the Publishers (Literature Cited, references 1-6) to quote or reproduce copyright material. Other references required to introduce the research and to discuss the data obtained are identified in the 6 publications resulting from the current study.

In conducting the research described in this report, the Principal Investigator adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences - National Research Council.

BODY OF REPORT

INTRODUCTION

It is known that species of Enterobacteriaceae contain a common antigen (CA), first described by Kunin et al., which is found in the ethanol-soluble (ES) fraction of supernatant fluids of 100°c-killed cultures (HKS) and modifies erythrocytes (RBC) for aggultination by CA antibodies. Ethanol soluble CA and not its precursor HKS which contains endotoxin (lipopolysaccharide), engenders humoral CA hemagglutinins in rabbits and human subjects. Moreover, the administration of CA or CA antiserum protects rabbits against experimental infection with Proteus mirabilis, a heterologous species which possess CA. However, the same CA preparation evokes minimal or equivocal antibody responses in the mouse and only transient protection, dependent on the mouse strain or challenge dose used. It is proposed that antigenic mimicry between animal tissue and enteric bacteria accounts for the seemingly anomalous immunologic indifference to CA noted. This concept was explored in mice and guinea pigs. Specifically, the purpose of the research program undertaken was to:

1. assess the immunogenicity of CA in the guinea pig as measured by cutaneous delayed-type hypersensitivity and humoral antibody response to homologous and heterologous sources of CA.

2. determine if protection against infectious challenge is evoked in mice immunized with isologous or heterologous RBC treated with CA.

3. assess strains of outbred, inbred, and gnotobiotic mice for tissue antigens cross-reactive with CA.

The results of the current study have been reported (1-5) and reviewed in part (6).

MATERIALS AND METHODS

Strains and Immunogens. Smooth strains of Escherichia coli 014,0111, and 086, Salmonella typhimurium and Pseudomonar aeruginosa were selected from our stock culture collection. The methods for the preparation of antigens and immunogens to include ES fractions have been described (1).

Animals. The following female animals were purchased from commercial sources: mice-(outbred Swiss white albino, inbred C57B4/6Ha, inbred DBA, and gnotobiotic Swiss white); Hartley albino guinea pigs; albino rabbits.

Tissue Extraction. Mice and rabbits were sacrificed with ether and their spleens, livers, or paired kidneys aseptically removed, minced, and homogenized in sterile phosphate buffered saline (PBS) (pH 7.3); subcultures on aerobic media were sterile. Supernate fluids of heated tissue hemogenates and subsequent ES fractions were employed to modify erythrocytes, inhibit CA hemagglutination, and immunize animals in accordance with procedures previously described in detail (2-5). Guinea pigs were tested for delayed-type hypersensitivity to CA by the method outlined earlier (1).

Measurement for Humoral Antibodies. Serum samples of immunized animals were assessed for CA antibodies by a micro-titer modification of the passive hemagglutination test.

Infectious challenge. One week after immunization with CA-treated RBC, mice were challenged intraveneously with 0.5 ml 10 LD_{50} S. typhimurium grown 16-18 hours in nutrient broth. The numbers of surviving mice were recorded each day until all mice had died; S. typhimurium was recovered in pure culture from heart's blood of random dying of dead mice.

Statistical Analysis. The significance of differences in survival rates between immunized and control mice was determined by the chi-square test. The significance of the cutaneous delayed-type hypersensitivity was calculated by a series of standard t-tests, using the skin reaction-site areas as raw scores. The results of these t-tests were confirmed by an analysis of variance

between the experimental and control groups.

RESULTS

Active immunization with CA elicits both humoral and cellular responses in guinea pigs as measured by serum hemagglutinin titers and cutaneous dialyzed hypersensitivity (Tables I-III, reference 1). Although minimal titers of serum antibodies are recorded, it is likely that manipulating the amount, route, or schedule of antigen administration would enhance these titers.

The administration of small amounts of CA on heterologous RBC significantly delays mortality of Swiss white albino mice challenged with the highly virulent S. typhimurium (Tables 1 and 2, reference 2). These findings complement and extent an earlier report that CA per se transiently protects mice against infectious death with S. typhimurium and recent evidence that it protects rabbits against renal disease induced by Proteus mirabilis.

Of the mouse-tissue preparations studied, only the ES fraction of the livers of Swiss white albino and C57BL/6Ha mice clearly showed capacity to inhibit CA-hemagglutination; titers were reduced approximately 98% (Table I, reference 3). These same preparations administered to rabbits failed to initiate a CA hemagglutinin response; however, 3 days after booster with CA, these identical animals demonstrated titers which were 3 or 4 fold higher than controls primed with PBS (Table II, reference 3). On day 30, 10 days after booster with PBS, rabbits primed with tissue-extract were sacrificed and their spleens removed, homogenized and examined for their capacity to form rosettes (RFC) with CA-treated RBC. Marked cellular activity, as noted by the significant numbers of RFC formed, was present in rabbits immunized with spleens or livers of either mouse strain (Table III, reference 3). Perusal of Tables I-III, reference 3, reveals that the antigenicity and immunogenicity of cross-reacting antigen in target organs of mice depend upon the mouse strain examined, the extraction procedure employed, and the in vivo testing method used.

The cross-reactivity recorded between mouse-tissue extracts and CA cannot be attributed to indigenous microbial flora, colonization, or to contamination from the

intestinal lumen; aseptic procedures were employed in excising each organ and subcultures invariably were sterile. Moreover, repeat experiments produced similar results (Tables I and II, reference 4). Significantly, as seen in the latter Tables, gnotobiotic mice (known to be free of microbial populations) clearly revealed organs with cross-reacting antigen. These data with tissue extracts of gnotobiotic mice were corroborated and extended in a detailed study separately recorded and published (5). It can be seen (Tables 1-3) that, according to the parameter of CA hemagglutination-inhibition, cross-reactive antigen is present in spleens, livers, and paired kidneys, but absent in colons. However, the identical preparations, including colons, primed rabbits to engender specific CA hemagglutinins after a single administration of CA. Also, spleens of these same rabbits were colonized with rosette-forming cells against sheep RBC treated with various enterobacterial sources of CA.

DISCUSSION

The results obtained in the current studies have been comprehensively discussed in the basic publications (1-5). The following narrative explores the biologic significance of CA, engendered antibodies, and mimicry between host tissue and CA.

Ethanol-soluble CA is stable to heat; immunogenicity and antigenicity are essentially unchanged after one hour at 100°C or 20 minutes at 120°C. In the small amounts required to engender CA antibodies, CA is not toxic, as shown by cytotoxicity studies with monolayers of guinea pig peritoneal macrophages and by mouse lethality tests (7). However, the possibility does exist that large amounts of CA may be slightly toxic to certain laboratory animals and to man, because ethanol-soluble CA is not completely devoid of contamination with trace amounts of endotoxin. Therefore, further purifications and characterization are required prior to classifying CA as an unequivocally inocuous immunogen.

In theory, an antigen shared by all species of Enterobacteriaceae, and capable of engendering humoral or cellular events against other bacilli within this family,

is of potential importance as a prophylactic vaccine. This thesis was first explored in mice. CA, extracted from culture of E. coli serotypes 014 or 0111, was administered intraperitoneally to Swiss white albino and C57BL/6Ha mice, which were subsequently challenged with 100 LD_{50} of S. typhimurium. Both mouse strains showed transient but statistically significant resistance, compared with uninoculated control animals infected at the same time; the Swiss white mice were the better protected. A major contribution regarding the protective role of CA was made by McLaughlin and Domingue (8). These investigators demonstrated that a majority of their rabbits administered CA resisted renal infection to retrograde challenge with Proteus mirabilis or to hematogenous challenge with E. coli 075; less than 20 per cent of the immunized animals developed renal pathology, while more than 50 per cent of the control rabbits had pyelonephritis. Moreover, none of the vaccinated animals had grossly visible abscesses. It is of further interest that none of the rabbits with CA hemagglutinin titers of 10,240 or more had pyelonephritis or pyelitis. Earlier, Frentz and Domingue (9) showed that rabbits with CA antibody titers higher than 640 were protected against hematogenous challenge with E. coli 06, whereas those with lower titers were not. It appears, therefore, that the efficacy of CA as a vaccine is determined by the specificity and titer of immunoglobulins engendered in selected animal models.

Based on Sephadex fractionation and susceptibility to 2-mercaptoethanol, CA antibodies belong to the 19S (IgM) class of immunoglobulins (10). At present, there is no explanation for the fact that these antibodies fail to agglutinate heterologous bacilli containing CA, although this antigen is present on the bacterial surface, as shown by fluorescent antibody studies (11). Nor will CA antibodies always precipitate CA; certain, but not all high titered CA antisera produce precipitin lines in gel (12). It is of academic interest that, independent of the source of CA employed as immunogen, CA antibodies are bactericidal for E. coli 014 but not for other enteric bacteria. Of biologic significance are reports that CA antiserum obtained from immunized rabbits and human subjects will opsonize heterologous enteric bacilli for phagocytosis by rabbit and human polymorphonuclear leukocytes, respectively. These observations suggest that CA antibodies may protect against enterobacterial infection. Indeed, preliminary studies with mice

have shown that CA antiserum (rabbit) provides slight to moderate, although temporary, protection against infectious challenge with S. typhimurium. More convincing evidence of protection by CA antiserum was documented in rabbits by Domingue et al. (13). These investigators showed that even low-titered CA antiserum provides significant protection against pyelonephritis if administered intravenously four hours prior to hematogenous challenge with Proteus mirabilis. The concept that the observed reduction in morbidity reflects directly the influence of CA antibodies is supported by control experiments in which CA antiserum failed to prevent pyelonephritis in animals challenged with Pseudomonas aerugenosa, which is devoid of CA. Moreover, removal of CA antibodies abolished the protective activity of the antiserum; rabbits challenged with P. mirabilis in this group displayed renal histophatology four weeks after infectious insult.

Currently, only limited information is available on the biologic significance of CA antibodies in healthy human subjects and patients with various infections. These antibodies are either absent or present only in relatively low titers in normal serum samples. A modest increase in titer has been observed in some, but by no means all, children with shigellosis, salmonellosis, or urinary tract infections caused by Gram-negative enteric bacilli (14). More recently, Neter et al. (15) showed that CA hemagglutinins were produced in significantly elevated titers in 86 per cent of the pediatric patients they had observed with pyogenic peritonitis. Whether these antibodies reflect the immunogeneic influence of the etiologic agent possessing CA or of a cross-reacting tissue antigen de-repressed during cellular infiltration, has not been determined. In this regard, it is of interest that a heightened humoral immune response to E. coli 014 has been demonstrated in patients with inflammatory bowel disease. Moreover, it has been proposed that stimulation by cross-reacting bacterial antigen, e.g. CA of E. coli 014, may be an important contributing factor for autoantibody formation in ulcerative colitis (16).

The occurrence of heterogenetic antigens in widely separated phylogenetic groups has been known since the turn of the century. Notably, the classic Forssman antigen has been found in bacteria, yeasts, nematodes, and in tissues of birds, fish, and mammals. Also, heterogenetic antigens other than Forssman exist between bacteria and mammalian species. In this regard, an immunologic relationship has been shown between E. coli 014 and colon of

the germ-free rat (16). Moreover, Rowley and Jenkin (17) propose that mice are susceptible to S. typhimurium infection because they possess an antigen in their tissue which cross-reacts with the pathogen; as a result, infection ensues because the animal fails to recognize the microorganism as foreign.

The premise that enterobacterial CA shares characteristics or determinants with animal or human tissue (16) requires exploration. Preliminary data obtained in our laboratory support this concept; an antigen which has antigenic or immunogenic attributes indistinguishable from CA has been identified in certain, but not all, target organs of inbred mice. The cross-reactivity observed cannot be ascribed to the indigenous microbial flora or to contamination during excision of the organ, because this antigen has also been recovered from certain organs of gnotobiotic mice. Moreover, subcultures of tissue homogenates were always sterile.

It is speculative whether or not CA will prove to be biologically significant in human medicine. However, the data and reports reviewed are in accord with the conjecture that this antigen and engendered antibodies should not be ignored as potential mediators of resistance to or recovery from Gram-negative bacterial infections. Many investigators endorse the thesis that the presence of CA in tissue accounts, in part, for the inexplicable spectrum of susceptibility, refractiveness, or equivocal response animal and human subjects have to enterobacterial immunization or infection.

LITERATURE CITED

1. Morgenstern, M.A. and Gorzynski, E.A., 1973. Immune Response of Guinea Pigs to Common Enterobacterial Antigen. Immunol. Commun. 2:495-506.

2. Gorzynski, E.A. and Krasny, S.A., 1975. Effect of Erythrocytes Treated with Enterobacterial Common Antigen on Experimental Salmonella typhimurium Infection of Mice. Med. Microbiol. Immunol. 161:163-170.

3. Gorzynski, E.A. and Krasny, S.A., 1975. Immunologic Mimicry Between Mouse Tissue and Enterobacterial Common Antigen. Immunol. Commun. 4:39-49.

4. Gorzynski, E.A., 1976. Cross-Reactivity Between Mouse Tissue and Enterobacterial Common Antigen (CA). Military Medicine 141:610-612.

5. Gorzynski, E.A. and Krasny,S.A.,1975. Cross-Reactivity Between Organ Extracts of Gnotobiotic Mice and Enterobacterial Common Antigen. J. of the Reticuloendothelial Society, 17:346-352.

6. Gorzynski, E.A., 1976. Biologic Significance of Enterobacterial Common Antigen (CA) and Engendered Antibodies. Military Medicine, 141:696-699.

7. Kessell, R.W.I., Neter, E. and Braun, W., 1966. Biological Activities of the Common Antigen of Enterobacteriaceae. J. Bacteriol, 91:465-466.

8. McLaughlin, J.C. and Domingue, G.J., 1974. The Immunologic Role of the Ethanol-Soluble Enterobacterial Common Antigen versus Experimental Renal Infection. Immun. Commun., 3:51.

9. Frentz, G. and Domingue, G., 1972. Effects of Immunization with Ethanol-Soluble Enterobacterial Common Antigen on in vivo Bacterial Clearance and Hematogenous Pyelonephritis. Proc. Soc. Exp. Biol. Med., 142:246-252.

10. Whang, H.Y., Yagi, Y. and Neter, E., 1967. Characterization of Rabbit Antibodies Against Common Bacterial Antigens and their Presence in the Fetus. Int. Arch. Allgerg., 32:353-365.

11. Aoki, S., Merkel, M. and McCabe, W.R., 1966. Immunofluorescent Demonstration of the Common Enterobacterial Antigen. Proc. Soc. Exp. Biol. Med., 121:230-234.

12. Whang, H.Y., Loza, U., Neter, E. and Milgrom, F. 1973. Gel Precipitation of Common Enterobacterial Antigen by its Antibody. Int. Arch. Allerg., 45: 905-914.

13. Domingue, G., Salhi, A., Rountree, C. and Little, W., 1970. Prevention of Experimental Hematogenous and Retrograde Pyelonephritis by Antibodies Against Enterobacterial Common Antigen. Infection and Immunity, 2:175-182.

14. Diaz, F. and Neter, E., 1968. Antibody Response to the Common Enterobacterial Antigen of Children with Shigellosis, Salmonellosis, or Urinary Tract Infections. Amer. J. Med. Sci., 256:18-24.

15. Neter, E., Kennedy, E.A. and Jewett, T.C., Jr., 1973. Antibody Response to Common Enterobacterial Antigen of Children with Pyogenic Peritonitis Infection, 1:12-16.

16. Perlmann, P., Hammarström, Lagercrantz, R. and Campbell, D., 1967. Autoantibodies to Colon in Rats and Human Ulcerative Colitis: Cross-Reactivity with Escherichia coli 0:14 Antigen. Proc. Soc. Exp. Biol. Med., 125:975-980.

17. Rowley, D. and Jenkin, C.R., 1962. Antigenic Cross-Reaction Between Host and Parasite as a Possible Cause of Pathogenicity. Nature, 193:151-154.

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